V. L. KUMARI* & L. L. NARAYANA*: A contribution to the embryology of Oxalidaceae

V. L. クマリ・L. L. ナラヤナ： カタバミ科の胚形成

When compared with the large number of species of the Oxalidaceae the number of taxa embryologically investigated is comparatively less. Davis (1966) reviewed the work of earlier embryologists like Jönsson (1800), Billings (1901), Hammond (1908), Samuelsson (1913), Schürhoff (1924), Mauritzon (1934), Noll (1935), Souèges (1939), Thathachar (1942), Govindappa and Boriah (1956) and Narayana (1962). Subsequenty Herr (1972), Herr and Dowd (1968) studied the development of the female gametophyte in *Oxalis corniculata*. The salient embryological features of the family are: secretory type of anther tapetum, simultaneous cytokinesis of pollen mother cells by furrowing, 2 or 3-celled mature pollen, bitegmic, anatropous, tenuinucellate ovules, Polygonum type of embryo sac ontogeny, nuclear endosperm, Asterad type of embryogeny and endospermic seeds with seed coat formed by both the integuments.

The present paper deals with the life history of *Oxalis latifolia* H. B. et K. and the development of the male and female gametophytes, endosperm and seed coat in *Oxalis pubescens* H. B. et K. and *Biophytum intermedium* Wight.

Materials and Methods The materials were fixed in F. A. A. Usual methods of dehydration, infilitration and embedding were followed. Sections were cut at 8—10 μm and were stained using crystal violet and erythrosin combination.

Observations

Microsporangium, Microsporogenesis and Male gametophyte: The development of the anther wall conforms to the dicot type. The male archesporium consists of a plate of 3-4 cells (Fig. 1). The differentiated anther shows the epidermis, endothecium, a middle layer and secretory tapetum (Fig. 2). The cells of the tapetum are uninucleate to begin with (Fig. 2) and become 2-nucleate by the time the pollen mother cells begin to undergo meiosis. The

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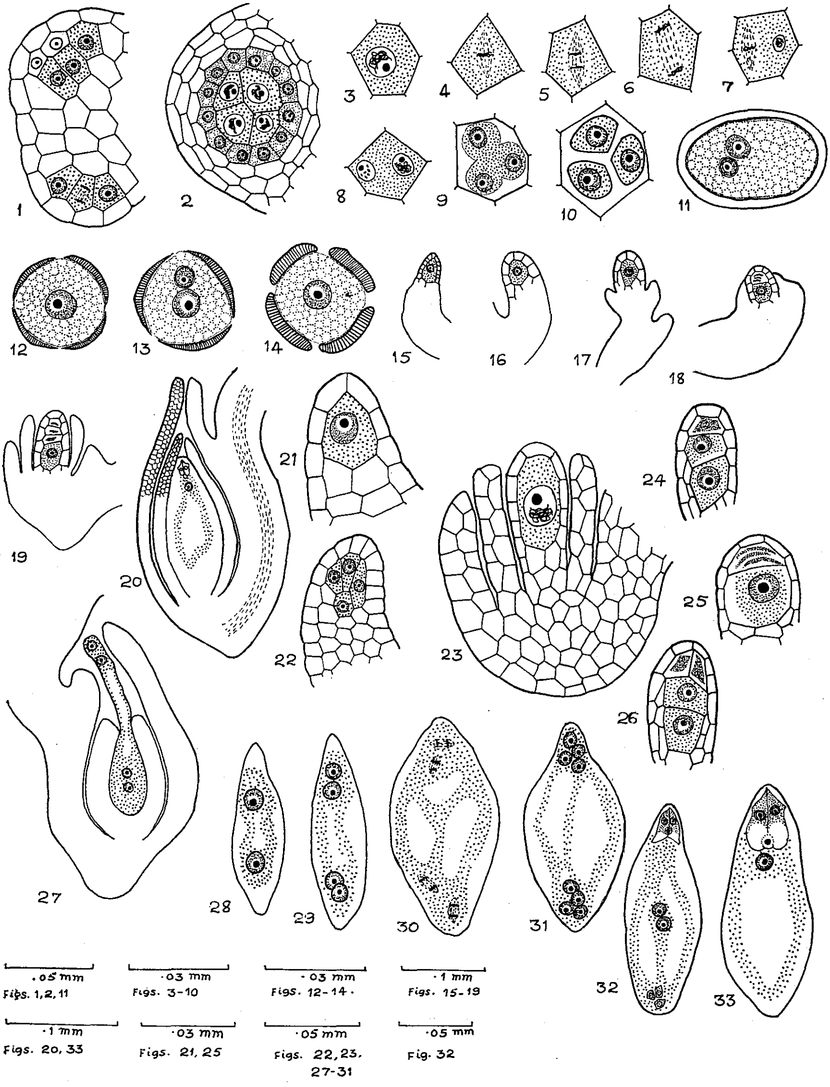
primary sporogenous cells undergo a few mitotic divisions before they function as the pollen mother cells. The pollen mother cells undergo meiosis forming four microspore nuclei within the mother cell wall (Figs. 3-8). Cytokinesis



takes place by simultaneous furrowing (Fig. 9). The pollen tetrads are arranged in tetrahedral manner (Fig. 10). The pollen grains are 2-celled at the shedding stage (Figs. 11, 13). They are spherical in *Oxalis* species (Figs. 12-14) and oblong in outline in *Biophytum intermedium* (Fig. 11). They are predominantly 3-colpate (Fig. 13), though in *Oxalis latifolia* and *O. pubescens* 4-colpate pollen grains were also observed (Figs. 12, 14).

Ovary and Ovule: The ovules are bitegmic, tenuinucellate and anatropous (Fig. 20). The ovule primordium arises on the placenta as a papillate outgrowth and grows upwards (Fig. 15, 16). The integumentary primordia are laid down by the time the megaspore mother cell becomes differentiated in the nucellus and grow upwards (Figs. 17-19). The integuments are free and are 3-cell thick along the antiraphe side (Fig. 20). The micropyle is formed by both the integuments (Fig. 20). The funicular vascular bundle terminates in the chalazal region (Fig. 20). There is no differentiation of an endothelium.

Megasporogenesis and Female gametophyte: The primary archesporium is hypodermal and usually single-celled (Fig. 21). In some ovules a multicellular archesporium was also noticed (Fig. 22). The archesporial cell directly functions as the megaspore mother cell (Fig. 23). The megaspore mother cell undergoes meiosis giving rise to a linear tetrad of megaspores (Fig. 19). There were also instances of triads and 'T' shaped tetrads (Figs. 24, 26). The lowermost megaspore enlarges during development (Fig. 25) and as a result of three successive free nuclear divisions gives rise to an eight nucleate embryo sac (Figs. 28-31) of the Polygonum type which shows the usual organization. The egg apparatus consists of an egg and two synergids which are

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Figs. 1, 10, 11, 18, 20, 21, 25, 32: *Biophytum intermedium*. Figs. 2-9, 12, 13, 15-17, 19, 22, 24, 26-31, 33: *Oxalis latifolia*. Figs. 14, 23: *O. pubescens*. 1. T.S. half anther showing archesporium. 2. T.S. anther lobe showing epidermis, hypodermis, middle layer, tapetum and microsporocytes. 3-8. Stages of meiosis in microspore mother cells. 9. Pollen mother cell showing cytokinesis. 10. Pollen tetrad. 11, 13. 2-celled pollen grains. 12, 14. Uninucleate pollen grains. 15-20. Stages in the development of ovule. 21. L.S. nucellus showing single-celled archesporium. 22. L.S. nucellus showing multicellular archesporium. 23. Ovule in L.S. at the megaspore mother cell stage. 24. Triad. 25. Linear megaspore tetrad. 26. 'T' shaped megaspore tetrad. 27. L.S. Ovule showing abnormal embryo sac. 28. 2-nucleate embryo sac. 29. 4-nucleate embryo sac. 30. 4-nucleate embryo sac. Note nuclei in division. 31. 8-nucleate embryo sac. 32. L.S. embryo sac showing egg apparatus, antipodals and polar nuclei. 33. Mature embryo sac pyriform in *Oxalis latifolia* and *O. pubescens* and hooked in *Biophytum intermedium* (Figs. 32, 33). The antipodals are ephemeral and the polar nuclei fuse to form the secondary nucleus just before fertilization. The mature embryo sac directly borders upon the inner integument.

In *Oxalis latifolia* at the 4-nucleate stage of the embryo sac, its micropylar end grows outwards through the micropyle and reaches the placental region. Only two such instances are met with (Fig. 27).

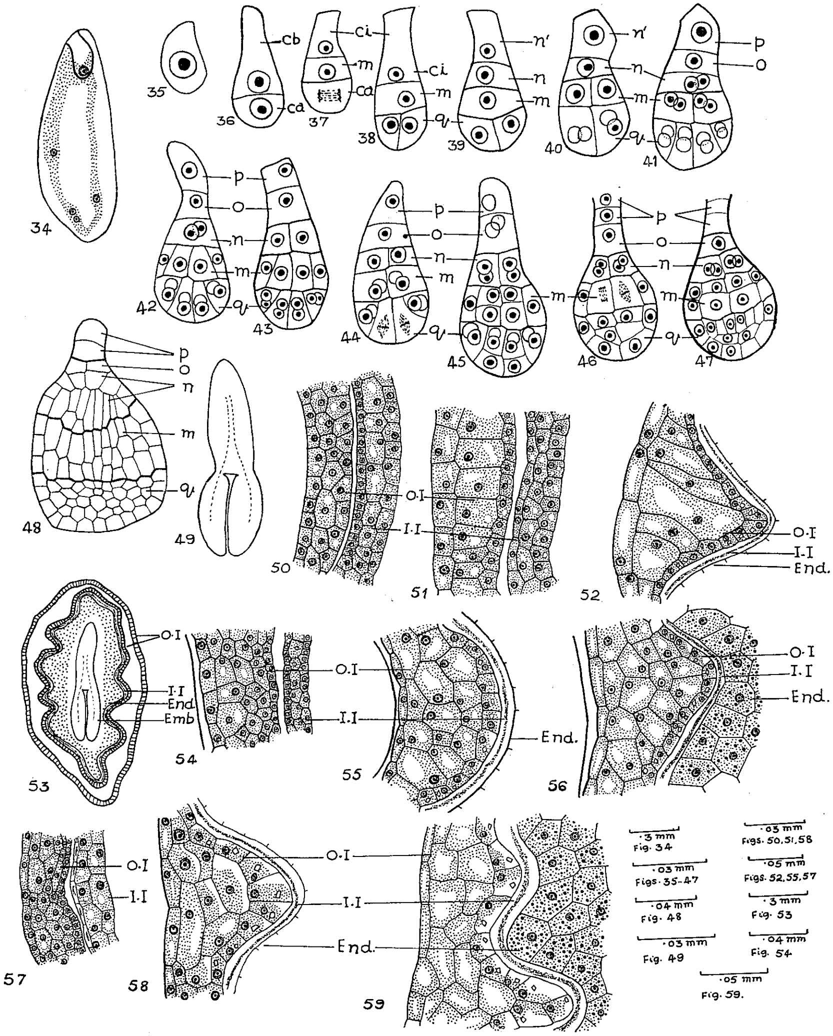
Fertilization and Endosperm: The primary endosperm nucleus divides in a free nuclear to give rise to a large number of free endosperm nuclei (Fig. 34). By about the time the embryo shows differentiation of the cotyledons, the endosperm becomes cellular and surrounds the embryo. In the mature seed the surface of the endosperm presents a wavy outline (Fig. 53). The seeds are endospermic.

Embryogeny: The first division of the zygote (Fig. 35) is transverse resulting in the formation of an apical cell *ca* and basal cell *cb* (Fig. 36). Cell *ca* divides vertically and cell *cb* transversely forming two superposed cells *m* and *ci* (Figs. 37, 38). The two of the terminal tier divide vertically at right angles to the previous division resulting in four circumaxial cells which constitute the tier *q* (Figs. 39, 40). The middle cell *m* divides vertically while *ci* divides transversely resulting in *n* and *n′* (Fig. 40). The cells of the tier *q* divide by obliquely vertical walls leading to the octant stage (Fig. 41). At about this time the cells of the tier *m* divide vertically resulting in four circumaxially arranged cells (Fig. 41). The cell *n* divides by a vertical wall and *n′* divides transversely forming *o* and *p* (Figs. 41-43). Next the cells of the terminal tier divide periclinally leading to the demarcation of the dermatogen layer (Figs. 42-45). At about this time the dermatogen initials are laid down in the tier *m* (Fig. 45). Sometimes the differentiation of dermatogen takes place earlier in the tier *m* (Figs. 42, 43). The cells of the tier *n* divide vertically to form four circumaxially arranged cells (Figs. 45-47). *o* also divides vertically (Figs. 45-47). As a result of divisions in the different embryonal tiers (Figs. 46, 47) the embryo attains a globular form (Fig. 48). At this stage the tier *o* consists of four circumaxially arranged cells and *p* divides transversely, forming two superposed cells, which function as a short suspensor (Fig. 48). The mature embryo is dicotyledonous (Fig. 49). The following schematic representation shows differentiation of the embryonic organs from the embryonic tiers.

ca—stem apex, cotyledons, hypocotyl
ZYGOTE :
cb—*m*—root initials
ci—*n*—root cap (in part)
n′—*o*—root cap (remaining part)
p—suspensor

Since both *ca* and *cb* of the 2-celled proembryo contribute to the formation of embryo proper, the embryogeny conforms to the Asterad type (Johansen, 1950) or the first period of embryogenic classification of Souèges, with the tetrad of the series A, Megarche type II (see Crété, 1963). Further, since the mode of embryo development in the present taxon follows a regular pattern, it is according to Group 4, series 15, variation 2, in the new approach to the classification of angiosperm embryos proposed by Periasamy (1977). The tier *m* gives rise to all the root initials and this is characterstic of the Oxalis variation (Johansen, 1950). Thus the embryogeny in *Oxalis latifolia* is essentially the same as in *Biophytum dendroides* (Noll, 1935) and *Oxalis corniculata* (Souèges, 1939).

Seed coat: The ovules are bitegmic and both the integuments are 3-cell thick (Figs. 50, 51). Only the outer integument takes part in the formation of the seed coat (Fig. 53). During post fertilization stages the outer epidermal cells of the outer integument become tangentially flattened (Figs. 52, 54-59) and the outer surface is covered by a thick cuticle (Figs. 54-56). The cells of the inner epidermis do not show any marked change in shape, except that they show calcium oxalate crystals during advanced stages of seed development (Figs. 52, 55, 56, 58, 59). In *Biophytum intermedium* the cells of the intervening layer undergo radial elongation in localized areas (Fig. 52). As a consequence of this the seed coat gradually assumes a wavy contour (Fig. 53). During the development of the seed, the inner integument becomes pressed between the growing endosperm and the outer integument (Figs. 52, 55, 56, 58, 59). The remnants of the inner integument can be made out even in the mature seed (Fig. 53). In *Oxalis latifolia* and *O. pubescens* the endosperm becomes ruminate due to the multiplication and inward enlargement of the middle layer of the outer integument (Figs. 54-59).



Figs. 34-49, 54-56: *Oxalis latifolia*. 50-53: *Biophytum intermedium*. 57-59: *Oxalis pubescens*. 34. L.S. embryo sac showing zygote and the nuclear endosperm. 35-48. Stages in the development of the embryo. For abbreviations see text. 49. Mature embryo. 50-52, 54-59. Stages in the development of the seed coat. Note the crystals in the inner epidermis of the outer integument. 53. L.S. mature seed showing seed coat, endosperm and embryo. Abbreviations: O.I: Outer integument. I.I: Inner integument. End: Endosperm. Emb: Embryo.

Discussion

The available information on the embryology of Oxalidaceae reveals that the family is embryologically homogenous.

The anther development in *Oxalis latifolia, O. pubescens* and *Biophytum intermedium* conforms to the dicot type of Davis (1966).

Plate-like archesporium in the anther, secretory type of anther tapetum with binucleate condition, furrowing method of cytokinesis of pollen mother cells, tetrahedral pollen tetrads, 2-celled condition of pollen at shedding stage, 3-colpate pollen grains, bitegmic tenuinucellate ovules, Polygonum type of embryo sac ontogeny, Asterad type of embryogeny are features common to the investigated taxa of the family (Davis, 1966; Govindappa and Boriah, 1956; Thathachar, 1942). While 3-colpate pollen grains are common, 4-colpate condition was also observed (present study). The development of endothelium reported by Thathachar (1942) in *Biophytum sensitivum, Averrhoa bilimbi* and *A. carambola* was not noticed in the taxa under the present study. The genus *Averrhoa* differs from other taxa of the family in the crassinucellate condition of the ovules.

Only the outer integument takes part in the formation of the seed coat, the inner integument becoming pressed during development. As a result of the radial elongation of the cells of the middle layer of the outer integument at certain places in *Biophytum intermedium* (present study) and multiplication and enlargement of the cells in certain areas in *Oxalis latifolia* and *O. pubescens*, the seed coat becomes markedly wavy and makes the endopserm ruminate. Thus, the ruminate endopserm in Oxalidaceae is according to the passiflora type of Periasamy (1962).

In essential embryological characters the Oxalidaceae resemble Geraniaceae, Balsaminaceae and Tropaeolaceae supporting their close relationship on embryological grounds.

According to Warburg (1938), Oxalidaceae, Geraniceae and Tropaeolaceae resemble one another in basic chromosome numbers ($x=7$ or 14 or $x=12$ or 14), chromosome morphology and chiasma frequency. According to Darlington and Wylie (1955) the basic chromosome number in the above three families is $x=7$, and according to Federov (1974) $2n=28$, 30, 42.

The family Limnanthaceae stands apart from these families and shows the following distinct embryological characters: unitegmic, tenuinucellate ovules, unusual type of embryo sac development, persistence of one of the synergids until the globular stage of the proembryo, synergid-like uppermost suspensor cell, single-seeded nutlet, backward extension of the cotyledons and pyramidal thickenings on the outer epidermis of the pericarp.

According to Corner (1976) "the small seeds of *Oxalis* may suggest affinity with Linaceae but the large fruit and more complicated seeds of *Averrhoa*, beyond the scope of that family, bear comparison with those of Caricaceae".

Hutchinson (1959) separated the genus *Averrhoa* from Oxalidaceae and raised it to the status of an independent family, Averrhoaceae. The genus *Averrhoa* (Thathachar, 1942) closely resembles Oxalidaceae in the embryological features. In the arborescent habit and crassinucellate condition of the ovules, the genus may be considered primitive in the family. It is therefore tentatively concluded that the removal of *Averrhoa* from Oxalidaceae, into an independent family Averrhoaceae is not justified. This is consitent with the views of Cronquist (1968) and Takhtajan (1967). Hence the genus *Averrhoa* can be included in the tribe Averrhoae under Oxalidaceae.

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カタバミ科の *Oxalis latifolia*, *O. pubescens*, *Biophytum intermedium* の花粉，胚嚢，胚乳，胚形成の過程を調べた。これらは殆んど同一の過程が観察される。花粉は3溝粒で散布の時期には2核であるが，ときに4溝粒のものが見られた。胚珠は倒惟で2珠皮があり，珠心は薄層である。珠心内に2—4個の大胞子が形成され，その最下位のものが胚嚢母細胞として発達する。胚乳形成は多核型である。胚形成は Asterad type の Oxalis variation にあたる。種皮は外胚珠のみから作られる。

こうした各器官の形成過程はゲンノショウコ科，ノウゼンハレン科，ツリフネソウ科などと一致する。カタバミ科から Averrhoaceae を特に区別する必要はないと考える。